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A VOLUMETRIC METHOD FOR ESTIMATING THE UNDENATURED SERUM PROTEIN IN MILK [1]

H. A. ANDERSON AND R. W. BELL
Eastern Utilization Research and Development Division, USDA, Washington, D. C.

SUMMARY

A rapid and simple method for determining the undenatured serum protein in milk, based on measurement of the volume of precipitated serum protein, is described. It involves the acid precipitation of the casein and the denatured serum protein and their removal by filtration. The soluble undenatured whey proteins in 7 ml. of filtrate are precipitated with 3 ml. of 10% phosphotungstic acid. Upon controlled centrifugation, the volume of the precipitate is obtained and converted to milligrams of whey protein nitrogen per gram of nonfat milk solids by means of a standard curve or the volume of precipitate may be used directly as the unit of measurement. Thus, the suitability of milk for a specific purpose is indicated.

The relation between milliliters of precipitate and milligrams of undenatured whey protein nitrogen as determined by Kjeldahl analysis has a correlation coefficient of 0.97. A standard curve is given which can be used routinely with the indicated equipment.

For some uses of milk, it is necessary to know the quantity of undenatured serum protein in the milk. Serum proteins are partially denatured during the manufacture of dry milk, particularly during the forewarming period. When nonfat dry milk (NFDM) is used in making cottage cheese, the amount of denaturation must be small if normal shrinkage of the curd and expulsion of whey are to take place. For bread-making, high-heat treatment of the milk with greater serum protein denaturation is desired, since it leads to improved baking quality. Yet, serum protein denaturation, in itself, has never been shown conclusively to be the change that improves the baking quality of the milk, and it is possible that some parallel change is responsible for the improvement (4).

There is no sharp division in undenatured whey protein nitrogen (UWPN) values to differentiate good and poor baking quality in milk. A suggested maximum value[1] for high-heat nonfat dry milk is 1.5 mg. per gram of nonfat solids as determined by the Harland and Ashworth procedure (1). A low-heat product suitable for cottage cheese manufacture should have a minimum value of 6.0 mg.

The method described in this paper is based on the measurement of the volume of undenatured serum protein precipitated by the addition of a suitable protein precipitating agent to a casein-free filtrate of milk. The range in volume of precipitate from a high-heat to a low-heat sample is shown in Figure 1.

MATERIALS AND EQUIPMENT

Reagents:

1. 10% acetic acid in distilled water: 10 ml. glacial acetic acid to 90 ml. water.

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[1] Standards Section, Dairy Division, AMS, U. S. Department of Agriculture. Washington, D. C.

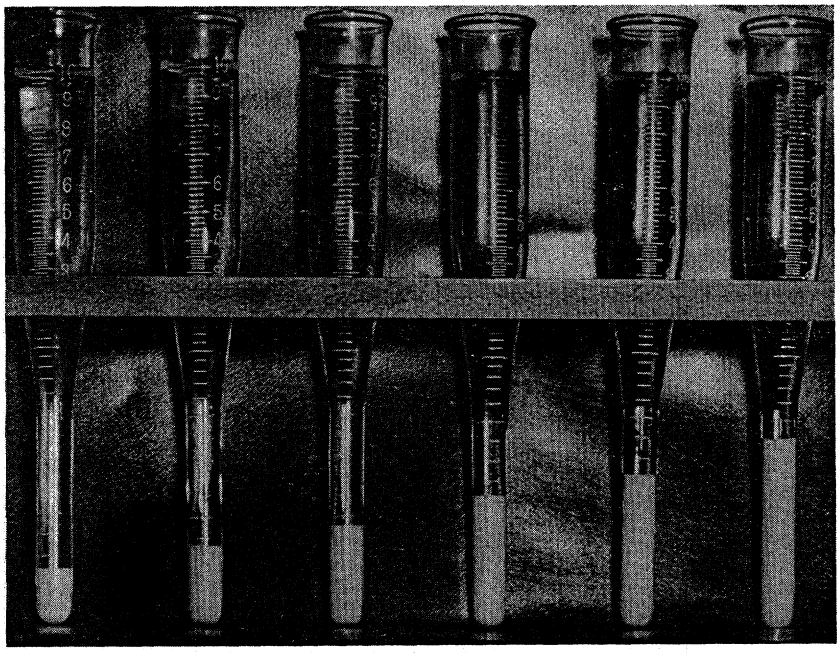

FIG. 1. The range in volume of precipitate from a high-heat to a low-heat sample.

2. 10% phosphotungstic acid in distilled water: 10 g. of phosphotungstic acid is diluted to 100 ml. Mild heat may be necessary to get all the phosphotungstic acid into solution.

Equipment:

1. Size 2 International centrifuge with a No. 240 head and No. 303 cups.
2. Tachometer for the centrifuge.
3. 100-ml. burette.
4. 300-ml. Erleneyer flasks.
5. Wrist-action shaker.
6. 10-ml. Kolmer centrifuge tubes.
7. 70-ml. delivery pipettes.
8. 100-mm. funnels.

EXPERIMENTAL PROCEDURE

1. A 10-g. representative sample of NFDM is weighed accurately and transferred to a 300-ml. Erlenmeyer flask. For dry whole milk the nonfat solids should equal those in 10 g. of NFDM. Ninety-three ml. of distilled water are added from a 100-ml. burette.
2. The flasks are attached by their necks to a wrist-action shaking machine and agitated for 10 min. at moderate speed. If a shaking machine is not available, samples should be mixed by swirling for 10 min.

3. Seven milliliters of a 10% solution of acetic acid are added slowly to the reconstituted skimmilk or milk with swirling of the flask. It is important that the swirling be not so forceful that the curd is broken and filtration is made more difficult. When acid is being added to the milk, mixing is necessary to distribute the acid uniformly, but harsh mixing produces a curd so fine that filtration is more difficult.
4. The precipitate should not be allowed to stand for more than 3 min.
5. The precipitate is removed by filtration, using a No. 3 Whatman filter paper. If the filtrate is not free of precipitate, it should be refiltered.
6. Seven milliliters of filtrate are pipetted into a 10-ml. Kolmer centrifuge tube.
7. Three milliliters of 10% phosphotungistic acid are added.
8. The suspension thus formed is mixed with a glass stirring rod until the particles are finer and will produce more uniform packing. Nonuniform packing is a potential source of error in the production of good replicates.
9. The tubes are centrifuged immediately in the following manner:
   The tachometer is lowered and the clock set for 25 min. The centrifuge is started and a speed of 500 r.p.m. is attained in the first 15 sec. In the second 15 sec. a speed of 1,000 r.p.m. is attained, and so on to 2,000 r.p.m. Then, the speed is maintained at 2,000 r.p.m. It is important that the centrifuge be allowed to come to a stop slowly.
10. The volume of precipitate is measured to a tenth of a milliliter and estimated to the nearest hundredth from the calibrations on the centrifuge tube.

PREPARATION OF THE STANDARD CURVE

To obtain a standard curve of maximum accuracy, undenatured whey protein nitrogen determinations were made by the Kjeldahl method on separate milk samples that had received different heat treatments. To do this, 30-g. samples of NFDM were reconstituted to a 10% solution with water, and 10% acetic acid was added to obtain a pH of 4.65. The necessity of using more concentrated solutions, in order to have enough precipitate for accurate determination of the volume of the whey protein, precluded the use of the dilute solutions of acetic acid (.017 M) and sodium acetate (.01 M) employed by Rowland (5). The buffering action of the milk produced the correct pH without use of sodium acetate. The casein-free filtrate was obtained by filtering through a double Whatman No. 1 filter paper. The soluble undenatured whey proteins were precipitated by adding one part 40% trichloroacetic acid to one part of milk (6). Kjeldahl nitrogen determinations were made on the noncasein and the nonprotein filtrates, using the catalysts employed by Rowland. The nonprotein nitrogen values were subtracted from the noncasein nitrogen values to obtain the UWPN. The milligrams of UWPN/g of NFDM are plotted against the milliliters of precipitate in Figure 2, and the ratio of unknown to control in Figure 3.

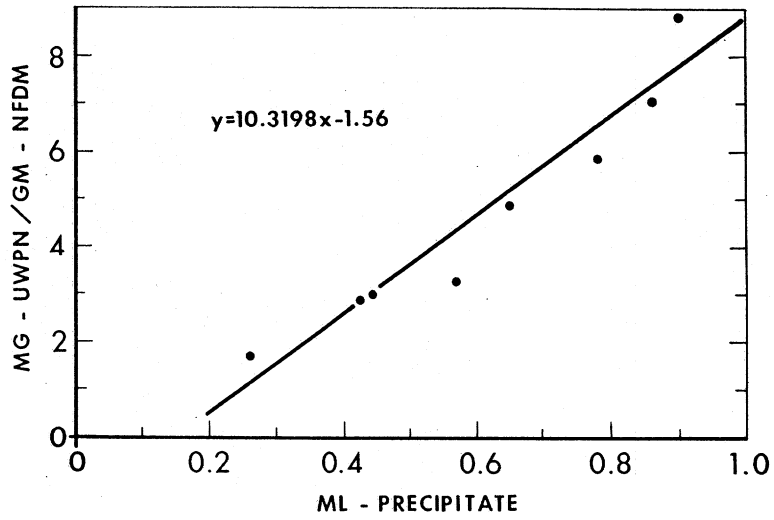

FIG. 2. Relation of the milligrams of undenatured whey protein nitrogen per gram of NFDM to the milliliters of precipitate.

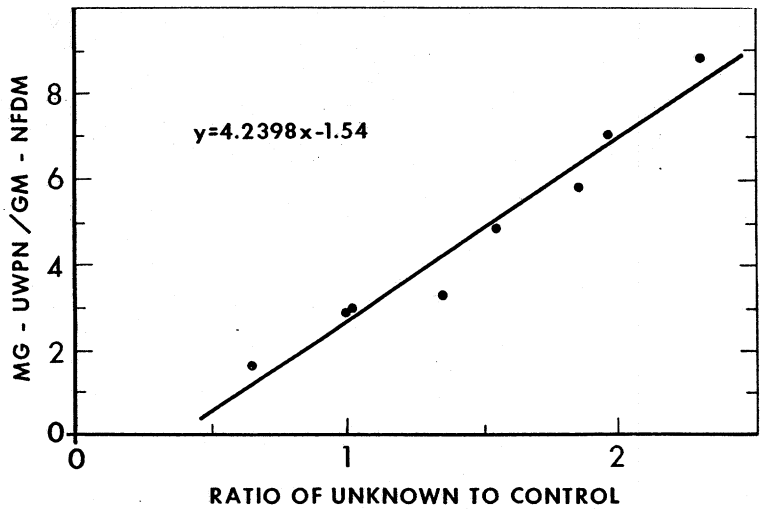

FIG. 3. Relation of milligrams of undenatured whey protein nitrogen per gram of NFDM to milliliters of precipitate using a control.

EXPERIMENTAL RESULTS

Tables 1 and 2 give typical results with milks of various heat treatments. Table 1 shows the correlation coefficient between mg. UWPN/g of NFDM (Column 2) as determined by Kjeldahl analysis, and milliliters of precipitate (Column 3) is + 0.97. The standard error for the determination of the values appearing in Column 3 is 0.01 ml. Table 2 shows the standard errors of four samples done in triplicate. Figure 2 shows the standard curve that was obtained by plotting the values in Columns 2 and 3 of Table 1. Each value represents the mean of eight determinations.

Sometimes it is more satisfactory to use the ratio of the milliliters of precipitate from a control milk to the milliliters of precipitate of the unknown. A reconstituted milk has relatively good keeping quality and so the same control can be used repeatedly. The reason for the ratio method of expressing the results is that the volume of the precipitate of the unknown varies as the control. This method of calculation gives a correlation coefficient of + 0.98 and a standard error of 0.03 between the ratio and the milligrams of protein nitrogen found in the casein-free filtrate by Kjeldahl analysis. For the control, the samples called "C (high-heat)" were used. Column 4 of Table 1 represents the ratio of the milliliters of precipitate of the control (the control had the same preparation as the unknown) divided into the milliliters of precipitate of the unknown. Each value represents the mean of four separate determinations. Values in Columns 2 and 4 of Table 1 are plotted in Figure 3.

It was found that fat did not interfere with the results as long as the calculations were based upon the measurement of the volume of precipitate from a casein-free filtrate. Therefore, if the weight of the samples of fluid or dry whole milk is based upon the weight of the nonfat portion, the results are comparable. This procedure is satisfactory for fluid whole and skimmilk and for whole and skimmilk powders.

DISCUSSION OF RESULTS AND COMPARISON WITH THE HARLAND AND ASHWORTH PROCEDURE

The present method, in which the volume of a precipitate is used to measure undenatured serum proteins, is based upon acid precipitation of casein at its isoelectric point, pH 4.60–4.70. The correct pH is obtained upon addition of 7 ml. of 10% acetic acid to 93 ml. of reconstituted skimmilk or fluid milk that contains the nonfat solids in 10 g. of NFDM. The Harland and Ashworth procedure is based upon the salting out of the casein and denatured whey proteins and their removal by filtration. The whey proteins remaining are precipitated with 10% HCl and the resulting turbidity is measured by means of a photoelectric cell. The per cent transmission is converted to milligrams of undenatured whey protein nitrogen by use of a standard curve.

The major difference in protein precipitants is that they precipitate different amounts and/or fractions of UWPN. This is shown in Tables 2 and 3. Table 2 shows the difference by Kjeldahl analysis between 10% phosphotungstic acid and 40% trichloroacetic acid in the precipitation of whey proteins. The 10% phos-

TABLE 1

Comparison of the milligrams of undenatured whey protein N/g of nonfat dry milk as determined by the Rowland method and the milliliters of precipitate of UWPN by the volumetric method

Sample No.	UWPN/g NFDM	Precipitate	Ratio to the volume from the control
	(mg.)	*(mg.)*	
6	1.65[a]	.26[b]	0.65[c]
C (High-heat)	2.87	.42	1.00
B5	3.00	.44	1.02
C4	3.30	.57	1.36
D5	4.86	.65	1.55
C2	5.84	.78	1.86
C1	7.06	.86	1.97
1	8.80	.90	2.30
Standard error		.01[d]	.03[d]

[a] Mean of duplicates.
[b] Mean of eight determinations.
[c] Mean of four determinations.
[d] Standard error of all determinations in column.

TABLE 2

Comparison of trichloroacetic acid and phosphotungstic acid as precipitants of UWPN

Precipitate	Standard error	UWPN/g NFDM by Kjeldahl Method		
(ml.)		*(mg.)*		
10% Phosphotungstic Acid		10% Phosphotungstic Acid	40% Trichloroacetic Acid	Difference
0.35[a]	.017	3.18[b]	2.76[b]	0.42
0.44	.012	3.71	3.27	0.44
0.67	.025	6.09	5.61	0.48
0.85	.018	8.73	8.43	0.30

[a] Mean of triplicates.
[b] Mean of duplicates.

TABLE 3

Comparison of results by the volumetric and the Harland and Ashworth methods on dry whole milk (DWM) and nonfat dry milk (NFDM)

Sample No.	Volumetric		Harland and Ashworth	Difference
	Precipitate	UWPN/g NFDM	UWPN/g NFDM	UWPN/g NFDM
	(ml.)	*(mg.)*	*(mg.)*	*(mg.)*
NFDM 1	1.00[a]	8.9	7.5[a]	1.4
NFDM 2	0.99	8.8	7.3	1.5
DWM 1	0.88	7.6	6.2	1.4
DWM 2	0.88	7.6	6.0	1.6
NFDM C	0.88	7.6	5.1	2.5
NFDM P	0.84	7.2	5.3	1.9
NFDM S	0.70	5.7	3.4	2.3
NFDM ADMI	0.40	2.6	1.3	1.3
NFDM 3	.34	2.0	1.2	0.8
DWM 3	.32	1.1	1.0	0.1

[a] Mean of duplicates.

photungstic acid precipitated more whey protein than the 40% trichloroacetic acid. Jenness (3), investigating the difference between salt precipitation (Harland and Ashworth) and hydrochloric acid precipitation (Rowland), found that salt precipitated a fraction that the acid did not, and that this fraction contained a large proportion of at least one of the heat-stable proteins formerly classified as proteose-peptone proteins.

Table 3 shows the difference between the volumetric and the Harland and Ashworth methods when milliliters of precipitate were converted by the standard curve to UWPN/g NFDM. There is too much variability between the two methods to establish an accurate conversion factor. This is particularly true of low-heat products (2).

An advantage of the volumetric method is that the volume (milliliters) of precipitate can serve directly as the unit of measurement, to indicate the relative amount of UWPN in NFDM. This eliminates any possible error resulting from an assumption that all undenatured proteins in the whey have the same nitrogen content.

The suggested maximum milligrams of UWPN/g of NFDM for good baking quality is 1.5 This corresponds to 0.30 ml. of precipitate. The suggested minimum for cottage cheese is 6.0 mg. This is equivalent to 0.73 ml.

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